

Abstract—We build on recent efforts to standardize maturation staging methods through the development of a field-proof macroscopic ovarian maturity index for Haddock (*Melanogrammus aeglefinus*) for studies on diel spawning periodicity. A comparison of field and histological observations helped us to improve the field index and methods, and provided useful insight into the reproductive biology of Haddock and other boreal determinate fecundity species. We found reasonable agreement between field and histological methods, except for the regressing and regenerating stages (however, differentiation of these 2 stages is the least important distinction for determination of maturity or reproductive dynamics). The staging of developing ovaries was problematic for both methods partly because of asynchronous oocyte hydration during the early stage of oocyte maturation. Although staging on the basis of histology in a laboratory is generally more accurate than macroscopic staging methods in the field, we found that field observations can uncover errors in laboratory staging that result from bias in sampling unrepresentative portions of ovaries. For 2 specimens, immature ovaries observed during histological examination were incorrectly assigned as regenerating during macroscopic staging. This type of error can lead to miscalculation of length at maturity and of spawning stock biomass, metrics that are used to characterize the state of a fish population. The revised field index includes 3 new macroscopic stages that represent final oocyte maturation in a batch of oocytes and were found to be reliable for staging spawning readiness in the field. The index was found to be suitable for studies of diel spawning periodicity and conforms to recent standardization guidelines.

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Staging ovaries of Haddock (*Melanogrammus aeglefinus*): implications for maturity indices and field sampling practices

Katie A. Burchard (contact author)¹

Francis Juanes²

Rodney A. Rountree³

William A. Roumillat⁴

Email address for contact author: katie.burchard@noaa.gov

¹ Department of Natural Resources Conservation
University of Massachusetts–Amherst
Amherst, Massachusetts 01003
Present address: Narragansett Laboratory
Northeast Fisheries Science Center
National Marine Fisheries Service, NOAA
28 Tarzwell Drive
Narragansett, Rhode Island 02882

² Department of Biology
University of Victoria
Victoria, BC, Canada V8W 3N5

³ Marine Ecology and Technology Applications, Inc.
23 Joshua Lane
Waqoit, Massachusetts 02536

⁴ Marine Resources Research Institute
South Carolina Department of Natural
Resources
217 Ft. Johnson Rd.
Charleston, South Carolina 29412

An important component of the assessment and management of any fish stock is quantification of the stock's productivity, which is a function of survival, individual growth, and reproductive success of a fish population (Wootton, 1998; Morgan, 2008). There are several factors that can be used to estimate the annual reproductive potential of a fish stock, including but not limited to sex ratio, age and size at maturity, spawning stock biomass, fecundity, and stock recruitment estimates where egg and larval viability are taken into consideration (Jennings et al., 2001; Morgan, 2008). Regular monitoring and data collection on reproductive potential, including estimation of spawning stock biomass, age and size at maturity, and fecundity, are dependent upon the use of reproductive maturity indices from a sample of the population (Tomkiewicz et al., 2003).

Because the ability to accurately determine reproductive maturity by macroscopic examination of the gonads alone is fallible, the validity of field reproductive indices has been

questioned (Hilge, 1977; Templeman et al., 1978; Saborido-Rey and Junquera, 1998; Vitale et al., 2006). Determination of maturation stages in the field has been criticized as not being dependable because different reproductive phases may appear similar during gross staging of the gonad. For example, estimates of spawning stock biomass or mean length at maturity will depend upon an accurate distinction between adult fishes with regenerating gonads and immature fishes (Forberg, 1982; West, 1990). Similarly, estimates of fecundity in determinate-spawning species, such as Atlantic Cod (*Gadus morhua*) and Haddock, require accurate identification of ovaries in prespawning stages (Murua et al., 2003). Therefore, it is important that the system used for determination of maturity stage is accurate and unambiguous (Brown-Peterson et al., 2011; Lowerre-Barbieri et al., 2011).

There have been considerable inconsistencies in the definitions of maturity stages of fishes among the existing indices in the literature. For example, O'Brien et al. (1993) defined

a female developing ovary as “a mixture of less than 50% yolked eggs and hydrated eggs”; however, according to Murua et al. (2003), the presence of hydrated oocytes indicate that the spawning process has begun and the gonad is in a “spawning” stage, where “oocytes are either in migratory nucleus stage or hydration stage.” This discrepancy between indices in the definition of a developing ovary could result in different estimates of fecundity in determinate-spawning species for which prespawning, when the most advanced oocytes in an ovary are in the late vitellogenesis stage, is the optimal phase in reproductive maturity for the collection of samples for accurate estimation of fecundity. If sampling is conducted before this stage, all oocytes destined to be spawned may not be developed and would be left out, and, as a result fecundity would be underestimated. If samples are taken from females that have already spawned, the number of eggs that have already been released cannot be detected, an outcome that also would result in an underestimation of fecundity.

Another important difference between the maturation indices of Murua et al. (2003) and O'Brien (1993) is the description of a resting ovary. The definition of O'Brien (1993) was based on a description by the NMFS (1989) and Kesteven (1960) and was similarly defined by Waiwood and Buzeta (1989), Tomkiewicz et al. (2003), and Vitale et al. (2006). All these authors described the resting maturity stage as occurring after the spent maturity stage. Conversely, Murua et al. (2003) described the resting stage as an in-between batch state occurring before the spent stage, when some hydrated oocytes from the previous batch may remain and further batches of hydrated oocytes are still to be produced. Therefore, there was a need for greater consistency in definitions and standardization in terminology of reproductive maturity stages of fishes. In a recent work by Brown-Peterson et al. (2011), a great deal of effort was invested in providing such standardization.

Although certain reproductive traits, such as maturity phases, are universal among teleost fishes, the temporal patterns of these traits vary among species (Lowerre-Barbieri et al., 2011). Incorporation of temporal components into standardized indices potentially could produce more accurate staging results for each species studied, as well as provide additional information on the reproductive success of a species. A recent study by Tobin et al. (2010), published after our sampling was completed in 2006–07, identified the timing and microscopic changes in maturation events of female Haddock as they transition from immaturity to maturity between summer and winter. That study provided evidence that Haddock commit to maturation by October or November with the existence of cortical-alveolar-stage oocytes in the ovaries. Knowledge of this maturation commitment can allow researchers to confidently identify females as either immature, skipped-spawner, or mature after November, improving estimations of spawning stock biomass.

Haddock is a batch-spawning species with group-synchronous ovary organization and determinate fecundity (Clay 1989; Murua and Saborido-Rey, 2003). This collection of reproductive traits is common in demersal Northwest Atlantic fishes, including but not limited to Atlantic Cod, Yellowtail Flounder (*Limanda ferruginea*), and Atlantic Halibut (*Hippoglossus hippoglossus*; see Murua and Saborido-Rey, 2003). The standard number of yolked oocytes immediately before the onset of spawning in a determinate-fecundity spawner can be considered equivalent to the potential annual fecundity of that fish (Murua et al., 2003). After the onset of spawning, the individual will hydrate several batches of yolked oocytes throughout the spawning season.

The purpose of our study was to develop a standard field-proof, macroscopic ovarian maturity index for Haddock that is suitable for use in studies of diel spawning periodicity (Anderson, 2011) and conforms to the recent standardization guidelines of Brown-Peterson et al. (2011). Diel spawning periodicity has been widely studied in marine fishes (e.g., Ferraro 1980; Walsh and Johnstone, 1992; Wakefield, 2010) and provides details on the chronology of reproductive processes in species. It has been suggested that diel spawning periodicity maximizes fish survival and reproductive success (Ferraro, 1980; Lowerre-Barbieri, 2011). In addition to support for the collection of field data on reproductive stages, we also wanted the index to provide guidance on sampling techniques for the collection of samples for laboratory analysis. First, a staging method developed from unpublished observations and a review of data published before our sampling in 2006–07 was used to stage female Haddock ovaries in the field. The resulting maturity index was then revised compared with a laboratory histological staging method similar to that of Tomkiewicz et al. (2003) for Atlantic Cod in the Baltic Sea. New stages were assessed to determine whether they could be used in future studies to examine diel patterns in spawning (Anderson, 2011). Finally, the relative strengths and weaknesses of both the field and laboratory approaches were assessed.

Materials and methods

Initial field and laboratory indices

A new field macroscopic ovarian maturity index for female Haddock was developed by building on previous published indices (Homans and Vladykoy, 1954; Robb, 1982; Murua et al., 2003; Brown-Peterson et al., 2011) and unpublished observations made in the field (Table 1). The index consists of 8 stages, progressing from immature to regressing. To move toward use of standard phraseology, the terminology follows Brown-Peterson et al. (2011). It differs from previously published indices with the addition of 3 stages that represent early to late progression of oocyte maturation (OM; Brown-

Table 1

Field index developed and used to stage the reproductive maturity of female Haddock (*Melanogrammus aeglefinus*) caught in the Gulf of Maine in 2006–07 during this study in which macroscopic methods in the field were compared with histological methods in the laboratory. OM=ooocyte maturation.

Stage	Abbreviation	Description
Immature	I	Ovaries small and firm, about 1/8 the volume of the body cavity. Membrane thin and transparent, gray to pink in color. Contents microscopic. Individual oocytes not visible to the naked eye.
Developing	D	Ovaries larger and plump, about 1/3 to 1/2 the volume of the body cavity. Membrane reddish-yellow with numerous blood vessels. Contents visible to the naked eye and consist of opaque eggs that give the ovaries a granular appearance.
Hydration stage 1	H1	Ovaries well developed, reddish-yellow in color, at least 2/3 volume of body cavity. Membrane opaque with blood vessels conspicuous. Contents consist mostly of yellow-looking oocytes with <25% of the ovary containing larger translucent oocytes. A batch of oocytes in the early stages of OM where oocytes start to hydrate.
Hydration stage 2	H2	Ovaries well developed, reddish-yellow in color, at least 2/3 volume of body cavity. Membrane opaque with blood vessels conspicuous. Visible surface of the ovary consists of 25–50% larger translucent oocytes. Further progression of a batch of eggs in OM.
Hydration stage 3	H3	Ovaries well developed, reddish yellow in color, at least 2/3 the volume of body cavity. Membrane opaque with blood vessels conspicuous. Visible surface of the ovary consists of 50–75% larger translucent oocytes. Ovaries may appear a little flabby, indicating the previous release of batch(es) of eggs. Final stages of the maturation of a batch of oocytes before a spawning event.
Ripe and running	RR	Ovaries very large, over 2/3 the volume of the body cavity. Contents consist of mostly large, translucent eggs. Eggs running freely with little to no pressure on the abdomen.
Regressing	S	Ovaries soft, and flabby, about 1/4 the volume of the body cavity. Membrane thick and tough, purplish in color, and bloodshot. Contents empty, few eggs remain, giving the gonad a patchy appearance.
Regenerating	RE	Ovaries small and firm, 1/6 the volume of the body cavity. Membrane thin but less transparent than an immature ovary, yellowish-gray in color. Contents microscopic, opaque.

Peterson et al., 2011) on the basis of the percentage of hydrated oocytes present (H1, H2, H3; Table 1, Fig. 1).

During observations of mature female Haddock ovaries, we noticed that many of them had varying numbers of hydrated oocytes. We did not find an ovarian maturity index in the literature that categorized the progression in percentage of hydrated oocytes in a gonad. We were interested in whether the increase in percentage of hydrated oocytes was detectable over time and whether these stages may aid in examination of diel reproductive periodicity (Anderson, 2011).

Hydration stage 1 (H1) is an ovary where a batch of oocytes is in the early phase of OM and when <25% of that ovary's visible surface contains translucent, hydrated oocytes (Table 1).

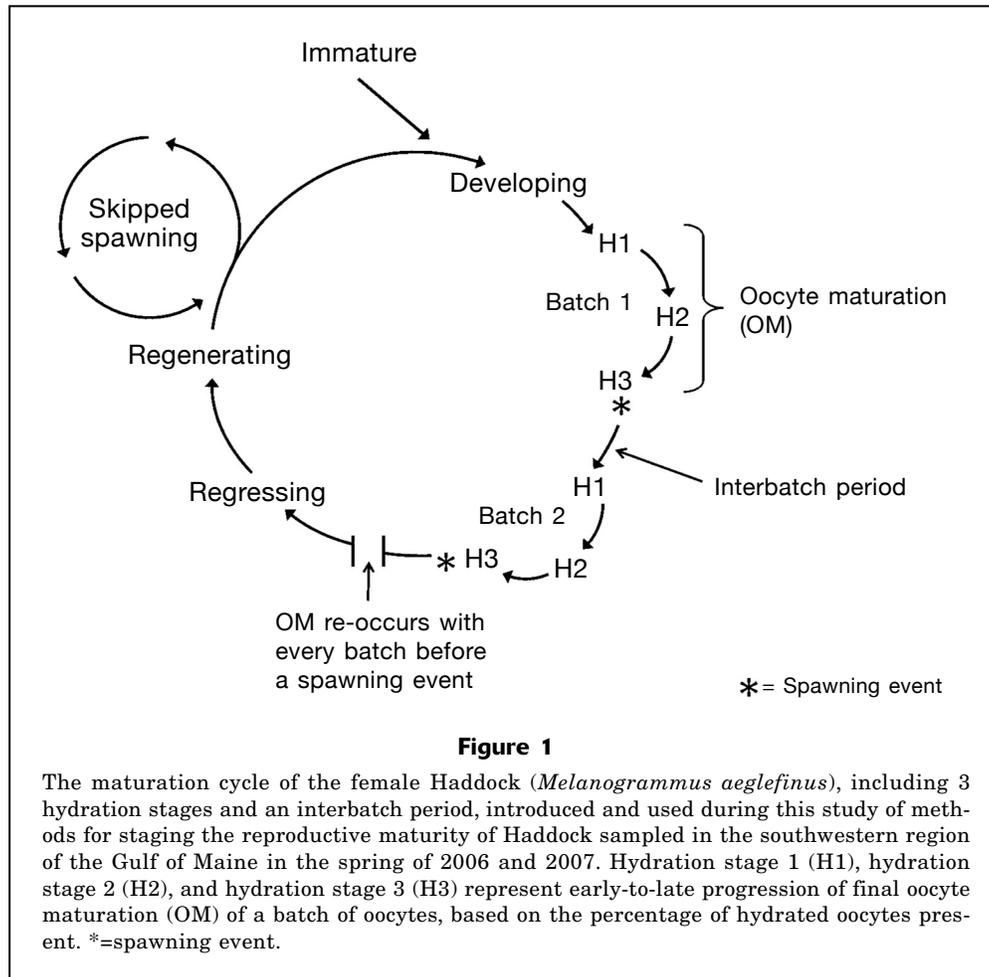
Hydration stage 2 (H2) is an ovary where a batch of oocytes is in the middle phase of OM and when 25–50% of that ovary's visible surface contains translucent, hydrated oocytes (Table 1).

Hydration stage 3 (H3) is an ovary with a batch of oocytes in a late phase of OM and when 50–75% of the visible surface of that ovary contains translucent, hydrated oocytes (Table 1).

We hypothesized that H1, H2, and H3 occur with each batch of oocytes before it is spawned (Fig. 1). The

index also includes for each stage: 1) a macroscopically derived ratio of ovary volume to body cavity volume, similar to the ratio of gonad cavity length to body cavity length that Robb (1982) included for some stages; 2) a physical description of the ovary membrane, as Homans and Vladykoy (1954) included for some of the stages; and 3) a grossly assessed oocyte development description, included by Homans and Vladykoy (1954), Robb (1982), and Murua et al. (2003) (Table 1).

The histological staging method was derived independently of the macroscopic ovarian maturity index (i.e., during analysis, field-based stages were not used by laboratory personnel in development of histological stages and vice versa), and it was based on previous work of Tomkiewicz et al. (2003), Roumillat and Brouwer (2004), and Brown-Peterson et al. (2011) (Table 2). To differentiate the processes of early versus later vitellogenic activity, 2 histological index stages (2.1 or 2.2) were used to define developing ovaries (Table 2). Because Haddock are classified as possessing determinate fecundity (Murua et al., 2003), all oocytes that will be spawned during the upcoming season develop during these 2 stages, leaving a group of primary oocytes as a reserve for the successive spawning season. However, the developing stages in the histological index (2.1 and



2.2) were grouped together as one developing stage (2.0) when the histology results were compared with the field results because those stages could not be differentiated by macroscopic examination. Three phases of spawning-capable (SC) ovaries were assigned in the histological index as 3.1, 3.2, and 3.3 to differentiate the process of early, middle, and late phases of OM: early germinal vesicle migration (GVM) and germinal vesicle breakdown (GVBD) (Table 2). The gross assessments of H1, H2, and H3 are based on morphologically distinct criteria that are corroborated by the histological sections that effectively separate these stages from each other (Table 2). Two histological index stages (4.1 and 4.2) were defined to categorize SC ovaries that showed evidence of recent ovulation with the presence of recent (4.1) or old (4.2) postovulatory follicles (POFs; Alekseyeva and Tormosova, 1979; Saborido-Rey and Junquera, 1998). POFs are ruptured empty oocyte casings left in the ovary after a spawning event (Table 2; Alday et al., 2010; Saborido-Rey and Junquera, 1998). If a sample contained POFs but also exhibited characteristics of another stage, the alternative stage was assigned with a note that the sample contained POFs (e.g., if a sample primarily contained oocytes in stage

3.1 but also contained POFs, it was assigned to the 3.1 stage).

Field sampling

Commercial fishing vessels were chartered for 25 dedicated survey trips in the spring of 2006 (15) and 2007 (10) to collect biological samples of Haddock in the southwestern Gulf of Maine (National Marine Fisheries Service Statistical area 514; Fig. 2). Surveys were based on a fixed station design with sampling where Haddock aggregations were known to previously exist. Sampling was conducted during the known spawning season of Haddock in the Gulf of Maine, between January and June (Brown, 1998). Haddock were identified in the manner used by Collette and Klein-MacPhee (2002).

Longlining was the preferred collection method for samples because few discards would result. Approximately 19 m of longline was set and retrieved 3 times at each sampling location over a 12-h period with the objective of having 2 consecutive trips represent sampling over a 24-h period (0100–0000 h; Table 3). Sets were conducted within specific 4-h time bins

Table 2

The reproductive maturity index developed and used in this study of staging methods for female Haddock (*Melanogrammus aeglefinus*) during histological analysis with analogous stages from the macroscopic field index. Histological definitions were based on criteria of Brown-Peterson et al. (Table 2 in 2011) CA=cortical alveolar; GVM=germinal vesicle migration; GVBD=germinal vesicle breakdown; NA=not applicable; OM=oocyte maturation; POF=postovulatory follicle; SC*=spawning capable, actively spawning subphase; Vtg1=primary vitellogenic; Vtg2=secondary vitellogenic; Vtg3=tertiary vitellogenic.

Histology	Stage	Macroscopic	Histological description
Immature	1.0	I	Small ovaries, only oogonia and primary growth oocytes present. Ovary wall thin, no muscle bundles evident.
Developing (early developing subphase)	2.1	D	Only primary and cortical alveolar oocytes present.
Developing	2.2	D	Primary growth, CA, Vtg1 and Vtg 2 oocytes present.
SC* early GVM	3.1	H1	Predominance of Vtg3 and early OM and beginning of GVM, yolk coalescence beginning. Few germinal GVBD oocytes observed, although some hydrated oocytes present.
SC* GVM	3.2	H2	Both early and late stages of GVM oocytes, obvious yolk coalescence occurring. Greater abundance of GVBD oocytes seen. Increased number of hydrated oocytes present.
SC* GVBD	3.3	H3	Predominance of GVBD oocytes, many with complete yolk coalescence. Many hydrated oocytes present—immediately before ovulation.
SC recent POF	4.1	NA	Many recent POFs present, showing few signs of degeneration. Otherwise advanced oocytes consist most noticeably of Vtg1–Vtg3 oocytes.
SC older POF	4.2	NA	Only older POFs present with advanced structural degeneration. Advanced oocytes consist of Vtg1–Vtg3 oocytes.
Regressing	5.0	S	Only spawning residue (old POFs) and primary growth oocytes remain in the ovary. Spawning effort for season ceased.
Regenerating	6.0	RE	Only primary oocytes remain in small ovary. Ovarian wall thickened, muscle bundles present.

(0100–0500 h, 0500–0900 h, 0900–1300 h, 1300–1700 h, 1700–2100 h, 2100–0000 h EST) to examine diel periodicity in reproductive maturity (Anderson, 2011). Each longline was fished with 150 to 400 circle hooks set 2 m apart for an average soak time of 2 h. The number of hooks fished per line on each trip was dependent on the success of catching Haddock that day. With the intent of sampling at least 50 Haddock from each longline set, the number of hooks was increased if the sample size was not reached or decreased if more fish than were needed were caught.

All Haddock were measured by fork length (FL, ± 1 mm) and examined externally for signs that indicated if they were in the ripe and running maturity stage (classified RR; Table 1). Ovaries were classified as RR when eggs were observed to be running freely from females with little pressure applied to the abdomen. The first 50 Haddock in each set were sacrificed to determine the stage of development of the gonads. If a fish ovary was observed to be ripe and running, its sex and maturation stage could be determined without excisions, and it was automatically classified as RR in the field. A subsample of the 50 sacrificed female Haddock that represented all reproductive stages from each longline set was labeled and reserved on ice. Fish from each of the following length bins were collected from each set if possible to have representation from as

many cohorts as possible: 30–40 cm, 40–50 cm, 50–60 cm, and >60 cm FL.

Laboratory methods

Samples were processed in the laboratory within 24 h of the end of each trip. Total weight (± 0.1 kg) and ovary weight (± 0.01 kg) of each individual were recorded. Macroscopic maturity stage of all samples was re-examined by the same field examiner. Digital photographs of whole ovaries were taken from a random subsample of each stage in the field index. To determine the accuracy of macroscopic maturity staging performed with our maturation index, histological analysis was conducted on tissue samples of a subsample of 169 ovaries from 1706 macroscopically classified fish representative of all 8 stages.

All histological tissue samples were taken from the forward right lobe of each ovary. It was assumed that this approach was appropriate because, according to Robb (1982), Haddock ovaries are homogeneous in structure throughout both lobes with oocytes present in various stages from the walls to the center of the ovary. Samples of 10-g tissue sections were fixed for at least 14 days in 10% neutral buffered formalin before they were transferred to 50% isopropyl alcohol. Samples were processed with standard histological procedures

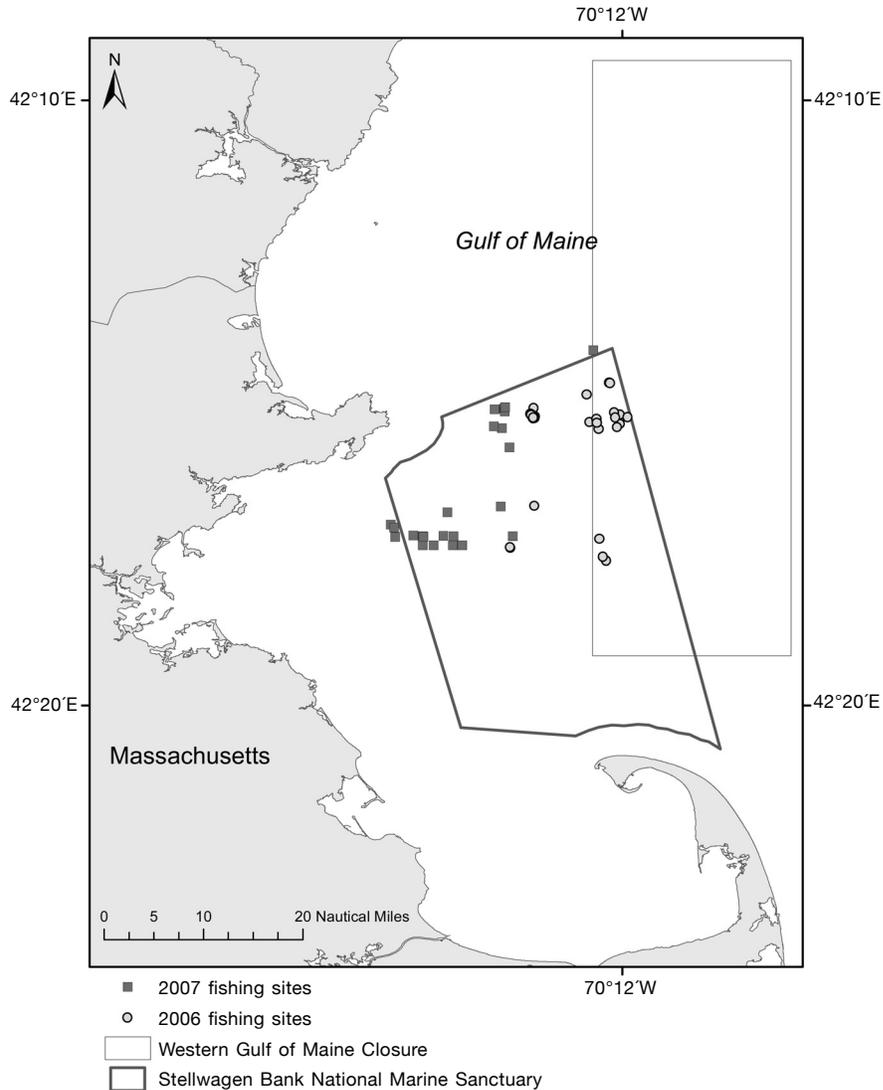


Figure 2

Map of the locations where mature female Haddock (*Melanogrammus aeglefinus*) were sampled in the southwestern region of the Gulf of Maine in the spring of 2006 and 2007 for for staging reproductive maturity.

(Humason, 1972) through a graded ethanol series, embedded in paraffin, and sectioned at 6 μ . Tissues were stained with Gill's hematoxylin and counterstained with eosin-Y. Ovary samples were classified by the occurrence of specific histological features that represent progressive oocyte maturation stages (Brown-Peterson et al., 2011) (Table 2). The most progressive feature observed in each sample was used to assign the appropriate stage. Photomicrographs were taken of a random subsample of stained tissue for each field index stage.

Statistical analysis

A contingency table was used to compare the results between the macroscopic staging methods used in the

field and the histological staging methods used in the laboratory (Table 4). The table cell where the 2 equivalent stages cross shows the number of samples for which the data from the 2 methods agreed. Because the 2 indices were developed independently, 2 different types of percent agreement were calculated. One type was derived by dividing the number of samples for which the 2 methods agreed by the field stage sample size (last row in Table 4). The second type of percent agreement was calculated by dividing the number of samples for which the 2 methods agreed by the histological stage sample size (last column in Table 4). We did not have enough observed frequencies in each cell to perform a chi-square statistical analysis.

Table 3

Dates of trips during which longlines were set and retrieved in the southwestern region of the Gulf of Maine in the spring of 2006 and 2007 to collect samples of female Haddock (*Melanogrammus aeglefinus*) over a 12-h period with the objective of having 2 consecutive trips represent sampling over a 24-h period.

24-h period	Year	Sampling dates
1	2006	3/12, 3/28, 3/31
2	2006	4/7, 4/10, 4/28
3	2006	4/30, 5/4, 5/8
4	2006	5/8, 5/16
5	2007	3/26, 3/31, 4/10
6	2007	4/10, 4/21, 4/24
7	2007	5/1, 5/22
8	2007	5/24, 5/30

Results

The results of each stage are formatted to explain both types of percent agreement as a function of each of the two staging methods. For each stage, the results of the

macroscopic field staging method are presented first, followed by the results of the histological laboratory staging method.

All 6 ovaries classified as immature (*I*) with the field index were also classified as the equivalent histological stage (1.0) in the laboratory. In contrast, all but 2 of the 8 samples classified as *I* (1.0) with the laboratory staging method were also classified as *I* with the field index (Table 4). Two samples classified as 1.0 in the laboratory were classified as regenerating (*RE*) with the field index.

Only 4 of the 9 ovaries classified as developing (*D*) with the field index were also classified as developing (2.0) with the laboratory staging method (Table 4). Two of the remaining ovaries classified as *D* with the field index were classified as the adjacent histological stage 3.1, and 2 samples contained early POFs (stage 4.1) and 1 sample contained late POFs (stage 4.2). In contrast, 7 of the 12 ovaries classified as 2.0 in the laboratory were classified as the adjacent *H1* with the field index, and 1 sample was classified as *RE*.

Twelve of the 32 ovaries classified as *HI* with the field index were also classified as the equivalent histological stage 3.1 (Table 4) in the laboratory. Seven of the ovaries classified as *H1* with the field index were

Table 4

Contingency table showing the results from the cross classification between the histological maturity stages (columns) and the field maturity stages (rows) in the indices used in this study of methods for staging the reproductive maturity of female Haddock (*Melanogrammus aeglefinus*). The gray squares represent where the cross classification is expected to have the highest frequencies of agreement. *n*=sample size; *PA*=percent agreement; *NA*=not applicable. If *NA* was used in place of *PA*, then that stage was not expected to agree with any of the opposing index stages.

		Maturity-index stages based on field examination									
		<i>I</i>	<i>D</i>	<i>H1</i>	<i>H2</i>	<i>H3</i>	<i>RR</i>	<i>S</i>	<i>RE</i>	<i>n</i>	<i>PA</i>
Maturity-index stages based on histological examination	1.0	6	0	0	0	0	0	0	2	8	75%
	2.0	0	4	7	0	0	0	0	1	12	31%
	3.1	0	2	12	0	1	0	1	0	16	75%
	3.2	0	0	2	21	2	0	4	0	29	72%
	3.3	0	0	5	9	22	17	2	2	57	39%
	4.1	0	2	1	1	0	0	0	0	4	NA
	4.2	0	1	5	2	0	1	0	0	9	NA
	5.0	0	0	0	0	0	1	4	16	21	19%
	6.0	0	0	0	0	0	0	1	12	13	92%
	<i>n</i>	6	9	32	33	25	19	12	33		
	<i>PA</i>	100%	44%	38%	64%	88%	NA	33%	36%		

classified as the adjacent histological stage 2.0, 2 ovaries were classified as 3.2, and 5 ovaries were assigned as 3.3. One H1-classified ovary contained early POFs, and 5 H1 ovaries contained late POFs. In contrast, 2 of the 16 samples classified as 3.1 in the laboratory were classified as the adjacent D stage with the field index, 1 sample was classified as H3, and 1 sample was assigned as regressing (S).

Twenty-one of the 33 ovaries classified as H2 with the field index were also classified as the equivalent histological stage 3.2 in the laboratory (Table 4). Nine H2-classified ovaries were classified as the adjacent histological stage 3.3. One ovary contained early POFs, and 2 ovaries contained late POFs. In contrast, 4 of the 29 ovaries classified as the 3.2 stage in the laboratory were classified as the adjacent field stages (H1 and H3), and 4 of those ovaries were classified as S.

The H3-classified samples were most frequently classified as the equivalent histological stage 3.3 ($n=22$; Table 4). Two H3-classified ovaries were classified as the adjacent histological stage 3.2, and 1 ovary was classified as 3.1. In contrast, 35 of the 57 ovaries classified as the histological stage 3.3 were classified differently with the field index, with most ovaries classified as H2 ($n=9$) or RR ($n=17$).

All but 2 of the ovaries classified as RR ($n=17$) in the field were classified as the histological stage 3.3 (Table 4). The 2 remaining ovaries were classified as the histological stages 4.2 and 5.0.

Four of the 12 ovaries classified as S with the field index were assigned the equivalent histological stage 5.0 (Table 4). Four additional ovaries classified as S with the field index were classified as the histological stage 3.2, and 2 ovaries were assigned as 3.3, 2 ovaries as 3.1, and 1 ovary as 6.0. In contrast, most of the 21 ovaries assigned to the histological stage 5.0 in the laboratory were classified as RE with the field index ($n=16$, 76%); however, 1 ovary was assigned as H3 (Table 4).

Twelve of the ovary samples classified as RE with the field index were classified as the equivalent histological stage 6.0 (Table 4). Sixteen samples classified as RE with the field index were classified as the adjacent histological stage 5.0 in the laboratory. Two additional samples classified as RE in the field were classified as histological stage 3.3, and 2 samples were classified as 1.0, and 1 sample was assigned as 2.0. In contrast, all but 1 of the 13 ovaries classified as histological stage 6.0 in the laboratory were also classified as RE with the field index.

A final composite ovarian maturity index was created on the basis of the findings from this study (Table 5). Visual characteristics for both the whole ovary and tissue sample were emphasized as was similarly done by Tomkiewicz et al. (2003) for Atlantic Cod in the Baltic Sea. The final index consists of 7 stages of ovary reproductive maturity distinguishable at sea. Table 5 includes for each maturity stage an image of the whole ovary, a photomicrograph of equivalent histological tis-

sue, and both a macroscopic and microscopic physical description of the ovary. Notes are included to aid the user in correct macroscopic identification of each stage. Sampling techniques for collection of tissue samples are also included for problematic stages. On the basis of comparison with the histological data, we concluded that H3 and RR field stages are identical and grouped them together as a single stage (H3). When we used this revised H3 field stage, 39 of the 44 ovaries assigned as H3 were assigned the equivalent 3.3 histological stage.

Discussion

The utility of the field-based staging method for the classification of fish reproductive maturity for fisheries management is dependent on its biological accuracy. The findings from this study highlight the problems of development of an accurate error-proof field ovarian maturity index on the basis of macroscopic observation. However, a comparison of field-based and histology-based staging methods of Haddock ovaries presented in this study revealed the need to revise the field staging methods to increase the accuracy of both staging methods. Although laboratory staging done on the basis of histology is inherently more accurate than any macroscopic field staging method, there was indication that field observations can reveal weaknesses in the laboratory approach because samples of the ovary taken for histology are not always going to be representative of the whole ovary. The strengths and weaknesses of both approaches for each maturation stage are discussed in the next sections, followed by recommendations for correct identification of each stage and a description of helpful sampling techniques for collection of tissue samples of problematic stages.

Immature stage

The I stage in the field index was equivalent to the 1.0 histological stage (Tables 1 and 2). The only stage mistaken for immature in the field was RE (Table 1). In both stages, the ovary was small and firm. The RE ovary appeared to be a little larger, less transparent, and grayer in color in comparison with the pink color of an immature ovary. However, in a young mature fish or late immature fish, these differences were less detectable. The imprecision in separation of immature and regenerating mature females also has been encountered in staging Atlantic Cod ovaries (Tomkiewicz et al., 2003). Comparison of the current mean length at maturity for Haddock with the size of the specimen may help support either maturity stage in the field, but this criterion should not be relied on because length at maturity can change over time (Saborido-Rey and Junquera, 1998; Tobin et al., 2010).

In this study, the smallest Haddock caught was 35.5 cm FL, larger than the mean length at maturity re-

corded for this species in the Gulf of Maine (34.5 cm; Collette and Klein-MacPhee, 2002). The gear type used in this study selected for larger fish, and we suspect that smaller fish avoided the longline hooks. Although to our knowledge skipped spawning (when a mature individual skips a year of spawning) has not been observed in Haddock, it is not uncommon in long-lived iteroparous fishes, including Atlantic Cod (Jørgensen et al., 2006; Rideout et al., 2006; Fig. 1). Therefore, we could not have assumed that a female was immature if it lacked signs of sexual maturity during the spawning season, as was assumed by Waiwood and Buzeta (1989) because there is the possibility that the fish had skipped spawning that year.

The use of microscopic analysis or histological examination of a tissue sample of the ovary was a reliable way to determine whether the ovary was immature or regenerating. Immature ovaries could be distinguished histologically from regenerating ovaries by the diameter of the primary oocytes (W. Roumillat, personal commun.). Immature ovaries contained primary oocytes that were equal in diameter, but regenerating ovaries had primary oocytes that varied in diameter. Additionally, the RE phase can be differentiated from the I phase by the following features: RE ovaries 1) have a thicker ovarian wall, 2) have more space, interstitial tissue, and capillaries around primary oocytes, and 3) have the presence of late-phase atresia and muscle bundles (blood vessels surrounded by connective and muscle tissue) (Brown-Peterson et al., 2011). Because of the selectivity of the fishing gear for larger-size fish and our limited sampling period, our study did not provide adequate data to fully resolve macroscopic differences between the RE and I stages. Further work should focus on differentiation of a regenerating ovary from an immature ovary with sampling conducted further into the summer with less size-selective gear. Proper identification of immature ovaries would greatly reduce the error in calculation of spawning biomass estimates and improve accuracy of estimates of length at maturity.

Developing stage

There was disagreement between D and early OM phase, H1 (Table 1). We observed that when a Haddock ovary began OM, some oocytes in the initial batch completed the process before others within the same ovulating batch. Although Haddock ovaries have been reported to be homogeneous in structure throughout all phases of maturity (Templeman et al., 1978; Robb, 1982), our observations indicate that it is not homogeneous in structure during this very early phase of OM (H1). This result is supported by Alekseyeva and Tormosova (1979), who reported that formation of batches occurs through asynchronous maturation of individual groups of oocytes. The histological staging method sometimes resulted in H1 ovaries being misclassified as D, likely because they were sampled during initial

OM of the first batch of oocytes for the season, when there were no histological characteristics present to indicate that prior batches had been spawned. Initial spawning H1 ovaries had so few fully hydrated oocytes (because of the asynchronous maturation of the batch) that collection of a small tissue sample from a central location was sometimes unsuccessful in representing all phases of oocytes present. As a single batch progresses through OM, evidence that spawning has been initiated becomes more obvious with GVM and yolk coalescence beginning in oocytes (Table 2; Lowerre-Barbieri et al., 2011). As the season progresses and the ovary initiates OM in later batches of oocytes, a H1 tissue sample could be distinguished from a D tissue sample by the presence of POFs.

The agreement between macroscopic and histological staging for D and H1 ovaries could be improved if the method used to take tissue samples from the ovary were modified. When ovaries are classified as H1 in the field, a larger tissue sample or samples should be taken from multiple places in the ovary to improve the accuracy of the histological results. Our observations demonstrate that determination of the maturation of an ovary based on histological examination alone may not always be accurate. To reduce staging errors based on histological analysis in future studies, it is recommended that each tissue sample be documented with a photograph of the whole ovary from which it was extracted and with an estimate of the percentage of hydrated oocytes observed on the visible surface of the ovary.

Three ovaries classified as D in the field contained POFs when analyzed histologically, and, by our definition, a D ovary could not have previously spawned that season (Table 1; Fig. 1). Therefore, those specimens had spawned at least one batch of eggs but had not yet hydrated oocytes for the next batch, and the decrease in volume of the ovary after spawning a prior batch of eggs was not evident in field observations. A closely related species, Atlantic Cod, begins to hydrate a batch of oocytes 1–2 days before spawning (Kjesbu, 1991). Final oocyte maturation in cold-water marine fishes with pelagic eggs generally lasts 1–2 days (Thorsen and Fyhn, 1996). Trippel and Neil (2004) reported that Haddock had a mean interval of 5.4 days between batches of released eggs, and Hawkins et al. (1967) and Alekseyeva and Tormosova (1979) reported an interval of 26–40 h. These findings combined indicate that there is an interbatch period between the spawning of a batch and the next batch that is beginning to hydrate, a period described by Murua et al. (2003) as the resting stage (Fig. 1).

Consequently, there was the possibility that a mature ovary could be incorrectly classified as D in the field if it was between ovulation events during this interbatch period. Therefore, we concluded that it is not always possible to be certain that an individual has begun spawning for the season on the basis of macroscopic observation alone and this uncertainty can pose

a problem for fecundity studies where ovary weight is used as a factor in determining fecundity. For the same reason, we also concluded that it is not possible to accurately stage an ovary as D by macroscopic observation alone. This issue poses a problem for studies that use gravimetric counting of vitellogenic oocytes and oocyte density to determine fecundity. The D stage, when the most advanced oocytes in the ovary are in the late vitellogenesis phase, is the optimal stage from which samples should be taken to determine fecundity. Therefore, we recommend that ovary samples be collected from fishes classified as D on the basis of macroscopic observations to confirm through microscopic or histological analysis that the ovary is in a prespawning state.

Hydration stages

A challenge in the use of the field index was the subjective evaluation of the percentage of hydrated oocytes in an ovary that was used to assign the consecutive H1, H2, and H3 stages. Therefore, histological samples were often assigned to a stage adjacent to the stage that was reported in the field. There were 5 instances where an ovary was macroscopically classified as H1 with the field index but microscopically classified as the histological stage 3.3. This difference in staging was likely due to some variation in individual and temporal batch fecundity (Trippel et al., 1998). However, this error was rare and the hydration stages were correctly staged consistently enough that we do not consider this misclassification problematic in identification of the correct hydration stage for the purpose of assessing diel reproductive patterns.

The histology-based laboratory staging method underestimated the H1 stage because the ovary typically appears to be heterogeneous during this stage and, therefore, was not adequately represented in the tissue samples. An H1-classified ovary could be incorrectly identified as D based on histological examination under these conditions. However, as an ovary matured further, the oocytes appeared to hydrate in unison and evenly throughout the ovary and nuclear migration and globule yolk coalescence became more evident. These criteria reduced the bias in the sampling method in later phases of H1 and eliminated it in later stages H2 and H3.

Histological analysis verified that H3-stage ovaries were in a state where the next batch of oocytes to be spawned were in final OM phase (GVBD), with most oocytes fully hydrated. This consistent result is important because both the field H3 and histological 3.3 stages can be confidently used to identify spawning readiness, and, therefore, we concluded that they will be well suited for use in studies of diel spawning periodicity in Haddock (Anderson, 2011) and other fishes.

Ripe and running stage

When the ovaries of RR females were examined macroscopically, they exhibited characteristics of the H3 stage. Furthermore, the tissue samples from these ovaries were classified as 3.3 (SC GVBD; Table 2) with histology-based methods. On the basis of results from the histological analysis conducted on ovaries classified as RR in the field and from the portion of the RR ovary full of hydrated oocytes during macroscopic observation, we decided to combine the RR and H3 field stages into a single stage in the final index (H3; Table 5).

Use of the RR field stage proved problematic because of the sampling method, and we recommend caution in its use in future studies. Homans and Vladykoy (1954) reported that female Haddock stop feeding during spawning—behavior that would make it difficult to catch actively spawning fish with baited gear and possibly result in an underestimation of RR females in the population. In addition, RR may be overestimated because of premature ovulation induced by stress or barotrauma. It is hypothesized that the barotrauma caused by forcing specimens to ascend to the surface from an average depth of 90 m during sampling can cause premature ovulation of hydrated oocytes. An increased level of cortisol in fishes is an indication of severe stress, but it is also involved in the natural process of ovulation (Billard et al., 1981; Wendelaar Bonga, 1997). The 2-h average soak time of the hooks in this study could have been enough time for the stress response to induce ovulation in an H3-stage fish before it landed on board the fishing vessel.

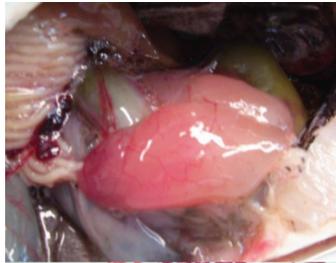
For the same reason, histological stage 4.1 may be overestimated, because the premature ovulation caused by barotrauma results in POFs appearing before they normally would. We concluded that it is difficult to catch a Haddock in the act of spawning, especially with baited hooks; therefore, use of H3-stage fish to estimate spawning readiness would be more accurate. However, the practice of macroscopically staging a RR Haddock through application of pressure to the abdomen and observation of the excretion of hydrated oocytes is a method that can be used to classify a female as spawning ready without need to sacrifice the fish.

Regressing stage

The S ovary stage was the most problematic for macroscopic identification. The regressing condition is particularly difficult to detect in a species such as Haddock with asynchronous development, where batches of eggs are spawned multiple times over a prolonged season (Hickling and Rutenberg, 1936; West, 1990). Species with determinate fecundity complete a spawning season by the maturation and spawning of the entire cohort of oocytes developed that year. When only a single batch of oocytes was left in the ovary to be spawned, it was termed “last spawn.” This stage was evident only during histological analysis. Of the ovaries classified

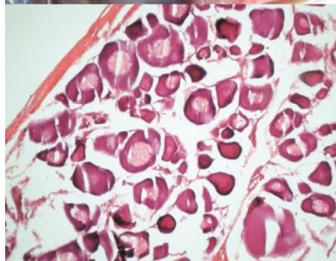
Table 5

The final female reproductive maturity index developed from findings with the macroscopic and microscopic method for staging the maturity of female Haddock (*Melanogrammus aeglefinus*).

**Immature (I)**

Macroscopic: The ovary is small and firm, and approximately 1/8 the volume of the body cavity. The membrane is thin, transparent, and gray to pink in color. Individual oocytes are not visible to the naked eye.

***Note:** This stage can look similar to a resting-stage ovary. Use of microscopic analysis or histology on a tissue sample of the ovary may be the only way to determine that the ovary is immature and not resting.

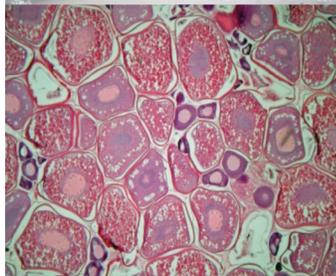


Microscopic: The ovary contains germ cells, oogonia, and primary oocytes. The ovary wall is thin and the primary oocytes vary little in diameter. No muscle bundles can be seen. The nucleus is relatively large with the most advanced oocytes having peripheral nucleoli (magnification 100×).

**Developing (D)**

Macroscopic: The ovary is plump and approximately 1/3 to 1/2 the volume of the body cavity. The membrane is reddish-yellow and has numerous blood vessels. The contents are visible to the naked eye and consist of opaque eggs, giving the ovaries a granular appearance.

***Note:** If hydrated oocytes are visible, the ovary should be classified as H1 (see the next stage below). Hydrated oocytes will be large in diameter and translucent in color. A large tissue sample should be taken from all ovaries macroscopically classified as developing and analyzed microscopically to confirm that postovulatory follicles are not present and that the ovaries are in a prespawning state. It may be helpful to document the tissue sample with a photograph of the whole ovary.



Microscopic: Primary and cortical alveoli oocytes, and primary and secondary vitellogenic oocytes are present. There is no evidence of postovulatory follicles (magnification 40×).

in the field as S, 58% ($N=7$) were classified as being in 1 of the 3 OM histological phases. The most plausible explanation for this result, other than observational error, is that these particular specimens were maturing the last batch of eggs to be spawned that season (last spawn) and the ovary at this point had lost its rigidity and, therefore, looked as though it was in the S stage. Last spawn was observed in 8 (5%) of the histological samples, 5 of which were classified as S in the field. Last spawn also was observed in Haddock in the North Sea (Alekseyeva and Tormosova, 1979). Near the end of the spawning season, the ovary can lose its

rigidity, although it still has 1–2 batches of oocytes to spawn and appears as S. The outside membrane thickens, which increases the difficulty of staging the ovary through examination of just the outside (Templeman et al., 1978). Staging on the basis of the flabbiness of the ovary alone is not recommended, and the inside of the ovary should be examined for hydrated oocytes. If any oocytes during final oocyte maturation (OM) remain, the ovary is most likely not in the S stage and could be in last spawn. Histological examination of a sample of an ovary can be an effective way to determine if an ovary is regressing.

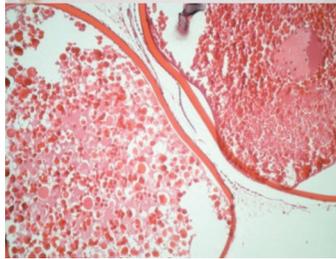
Table 5 continued



Hydration stage 1 (H1)

Macroscopic: The ovary is well developed, reddish-yellow in color, and approximately 2/3 the volume of the body cavity. The membrane is opaque and has prominent blood vessels. The contents consist mostly of yellow-looking oocytes and <25% of the ovary contains large translucent (hydrated) oocytes.

***Note:** In the early phase of the H1 stage, the ovary is not visually homogeneous and hydrated oocytes can be unevenly scattered throughout. If microscopic analysis will be conducted on a subsample, take care to obtain a representative tissue sample that includes translucent, hydrated oocytes. Document with a photograph of the whole ovary if possible.



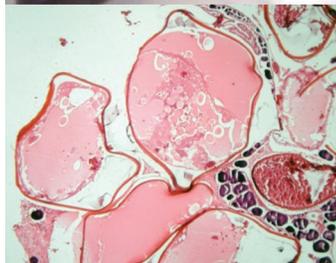
Microscopic: There is a predominance of tertiary vitellogenic oocytes, with many oocytes showing oocyte maturation, germinal vesicle migration and germinal vesicle breakdown. A small percentage of oocytes (<25%) will have completed oocyte maturation and are hydrated. Postovulatory follicles may be present (magnification 100×).



Hydration stage 2 (H2)

Macroscopic: The ovary is well developed, reddish-yellow in color, and approximately 2/3 the volume of the body cavity. The membrane is opaque with blood vessels conspicuous. The visible surface of the ovary consists of 25–50% of large translucent oocytes.

***Note:** There are gradients between the consecutive H1 and H2 stages as well as the H2 and H3 stages, where it is difficult to assign one or the other stage. In these cases, the ovary is at a state where it is either close to entering the H2 stage or close to advancing to H3. In both cases the ovary is near if not in an intermediate phase of final oocyte maturation and may be accurately classified as H2.



Microscopic: There is a predominance of oocytes showing germinal vesicle migration and germinal vesicle breakdown. Approximately 50% of the advanced oocytes are hydrated. Postovulatory follicles may be present (magnification 40×).

Regenerating stage

The histological results for RE stage ovaries reflected the difficulty in distinguishing between a regenerating and regressing ovary in the field, with 46% of the ovaries classified as RE in the field assigned as S during histological analysis. The plausible explanation for this result is observational error. As the ovary progressed into the RE stage, it became easier to differentiate from the S stage, but, because of the short sampling period, it was difficult to differentiate between the 2 stages during the time when regenerating fish were captured. For future studies, we recommend that sampling be conducted from well before to well after the

known spawning season and that a photograph of each ovary be taken for comparison with histology-based staging results. Such documentation of the changes observed in different phases, from spent to regressing, could improve the ability to distinguish between these 2 stages. However, extension of the sampling period too far into the fall and winter may make it more difficult to distinguish the D and RE stages from spawning stages (Tomkiewicz et al., 2003). Histological examination of a sample of an ovary was an effective way to determine if an ovary was in the RE stage.

If a regenerating ovary was observed from a fish near or larger in size than the mean length at maturity during the peak spawning period, it is possible that

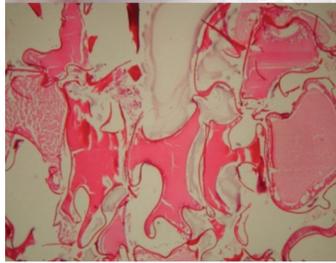
Table 5 continued



Hydration stage 3 (H3)

Macroscopic: The ovary is well developed, reddish-yellow in color, and approximately 2/3 the volume of the body cavity. The membrane is opaque with blood vessels conspicuous. Greater than 50% of the visible surface of the ovary consists of large translucent oocytes.

Microscopic: There is a predominance of oocytes showing germinal vesicle migration and germinal vesicle breakdown. Greater than 50% of the advanced oocytes are hydrated. Postovulatory follicles may be present (magnification 40×).

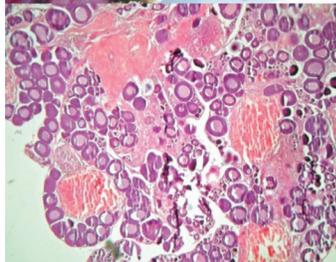


Regressing (S)

Macroscopic: The ovary is soft and flabby and approximately 1/4 the volume of the body cavity. The membrane is thick and tough, purplish in color, and bloodshot. The inside of the ovary is almost empty and few oocytes remain, giving the gonad a patchy appearance.

***Note:** Toward the end of the spawning season, the ovary loses its rigidity although it still has 1–2 batch(es) of oocytes to spawn. Staging should not be based only on the flabbiness of the ovary, and the ovary should be inspected internally. The ovary is likely not yet spent if any hydrated oocytes remain.

Microscopic: An abundance of postovulatory follicles are present. Oogonia and primary oocytes are evident. The ovary wall is thick, and muscle bundles are visible (magnification 40×).



it spawned much earlier that season or skipped that year's spawning season (Fig. 1). One mature regenerating female was observed during the peak of the spawning season. Skipped spawning is a response to various physiological and ecological conditions (Jørgensen et al., 2006) and often a trade-off between present reproduction and survival for future reproduction (Bull and Shine, 1979; Rideout et al., 2005). Because it is not possible to determine the existence and frequency of skipped spawning and its effect on recruitment, it is difficult to determine spawning stock biomass and, hence, difficult to conduct stock assessments and manage such species (i.e., stock-recruitment models may overestimate recruitment and underestimate survival; Rideout et al., 2005).

Postovulatory follicles

POFs were commonly found in ovary samples classified as H1, H2, H3, and S in the field, but these POFs often were in various phases of atrophy. The observation of early and late phases of POFs in the same ovary indicated that POFs from the 2 previous batches still existed during the OM of the next batch to be spawned (Table 2). Evidence indicates that the complete atrophy of a POF in Haddock could take up to 10 days, considering that Haddock have an average interval of 5.4 days between spawned batches (Trippel and Neil, 2004), and that final oocyte maturation in marine fishes with pelagic eggs generally lasts 1–2 days and ends with ovulation (Thorsen and Fyhn, 1996). The atrophy

Table 5 continued

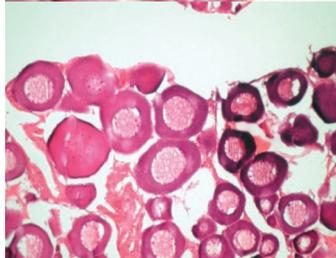


Regenerating (RE)

Macroscopic: The ovary is small and firm, and approximately 1/6 the volume of the body cavity. The membrane is thin but less transparent, yellowish-gray. Contents are microscopic, opaque.

Microscopic: The ovary wall is thick. There is often indication of past spawning with remnants of unabsorbed material. The ovary contains primary oocytes that vary largely in diameter (magnification 100×).

*Note: If a resting ovary is observed from a fish greater in size than the mean length at maturity during the peak spawning period, then it is probable that the fish skipped that year's spawning season.



of POFs occurs for the Spotted Seatrout (*Cynoscion nebulosus*) in 24–36 h in water temperatures $>2^{\circ}\text{C}$ (Roumillat and Brouwer, 2004) and for the Northern Anchovy (*Engraulis mordax*) in 48 h at 19°C (Hunter and Macewicz, 1985). The atrophy of Haddock POFs may take much longer because this species prefers to spawn in cold temperatures ($4\text{--}7^{\circ}\text{C}$; Overholtz, 1987)—an actuality that may be widespread in boreal fishes. The slow degeneration of POFs in cold-water species is supported by Brown-Peterson et al. (2011) and noted by Saborido-Rey and Junquera (1998).

Aging of POFs has been used in other species to determine spawning frequency or duration of time since the female last spawned a batch of eggs (Hunter and Macewicz, 1985; Roumillat and Brouwer, 2004). No definitive information on diurnal timing of spawning was clear from our inspection of Haddock POFs because none of them appeared to have been very recently created. Fish collections were concentrated in an area where active spawning took place, and those Haddock that had finished spawning may not have been available for capture. Observation of many ovaries in spawning condition that also showed many phases of POF atrophy indicated that these residual tissues had a very slow rate of atrophy and were of little use in making accurate assessments of diel timing of ovulation. A more advanced study of aging POFs in cold-water species similar to the studies done for clupeiforms by Alday et al. (2010) and Haslob et al. (2012) is needed and would increase our knowledge on the timing of spawning in cold waters.

There were no equivalent field index stages for the histological stages 4.1 and 4.2. Samples classified as 4.1 or 4.2 were typically assigned to an ovary in a state

between the last batch of oocytes spawned and the next batch to be spawned, a state that we did not attempt to identify in the field. In ovaries of this state, no oocytes for the next batch had yet progressed to OM and the only oocytes present were in a vitellogenic developed phase equivalent to the resting stage described by Murua et al. (2003). We found that this stage was not easily or accurately ascertainable through macroscopic observation of the ovary. A trained eye may be able to recognize a degree of flaccidity of an ovary that has spawned already. Many of the ovaries assigned as 4.1 or 4.2 exhibited characteristics of an ovary that was classified as the D stage in the field. The overestimation of the D stage in this study indicates the need to conduct histology on a subsample of ovaries classified as D stage in the field to assure there is no indication, on the basis of the presence of POFs, that females thus classified have started spawning that season.

Conclusions

Working independently, we came to the same conclusion as Brown-Peterson et al. (2011): standardization of maturation staging methods and terminology are needed. Our study confirms the importance of these efforts but extends them with the development of a new ovarian maturity index specifically for examination of diel spawning periodicity while using the maturation terminology established by Brown-Peterson et al. (2011).

Comparison of macroscopic and microscopic observations of ovaries helped us to improve the initial field index and sampling methods, as well as to provide useful insight into the reproductive biology of Haddock.

Noting the apparent longevity of POFs helped us understand the duration and cyclical process of OM in this species and potentially other boreal or cold-water fishes. Because reproductive maturation occurred over a prolonged period of time, OM occurred throughout 3 distinct field stages (H1, H2, and H3) and histology stages (3.1, 3.2, and 3.3). This finding supports the conclusion of Alekseyeva and Tormosova (1979) that Haddock exhibits asynchronous maturation of individual groups of oocytes. We believe that the asynchronous maturation of oocytes in a batch results in heterogeneous ovaries during early phases of OM and can lead to misclassification of H1 ovaries as D stage in the field. However, Robb (1982) and Templeman et al. (1978) previously reported that Haddock ovaries are homogeneous in structure throughout all phases of maturity. Studies of follicle size-frequency distributions throughout OM are needed to confirm our observation of apparent heterogeneity of ovaries during early maturation to clarify how future studies should be modified to ensure accurate staging in the field and laboratory.

Additional work should be focused on differentiation of a regenerating ovary from an immature ovary. This differentiation is the most important distinction in determination of maturity or reproductive dynamics of a stock because of the use of these numbers in estimation of spawning stock biomass.

The timing of the sampling in this study, although restricted, was focused around the known spawning season of Haddock in the Gulf of Maine. This focus likely increased the reliability of staging SC fish because the closer in time to the spawning season the more developed the ovary becomes, as was observed by Tomkiewicz et al. (2003). Alternatively, reliability in staging SC fish in the fall and winter is tenuous because ovary development is just beginning (Tomkiewicz et al., 2003). Therefore, the optimal time to collect data to be used to estimate spawning stock biomass should span across the spawning season, and we caution against the use of SC data collected off season in estimation of spawning stock biomass.

It is anticipated that the revised ovarian maturity index (Table 5) presented in our study will be useful to Haddock resource managers. The H2 and H3 stages appear to be useful indicators of spawning readiness for Haddock ovaries in the field. We suspect that the progression of OM is detectable in other boreal species with the same reproductive traits as Haddock and that the later stages could also be used to examine diel periodicity in these species. Although this index was developed for studies on diel reproductive periodicity, we feel it would also be useful for study of other short-term temporal reproductive patterns related to tidal, lunar, or solar zenith cycles. The revised field index includes pointers to help users stage ovaries and take appropriate samples (Table 5). Although this revised field index will improve accuracy in the determination of the maturity stage of Haddock in the field, evidence has shown that field indices alone may not be enough to

correctly classify a fish in problematic stages. However, the observations in our study also demonstrate that determining the maturation of an ovary by histological examination alone may not always be accurate, highlighting the importance of field staging. In addition to field staging with the index presented here, appropriate tissue samples should be collected and analyzed microscopically or histologically to verify problematic stages, especially when field data are used in assessment and management of a fish stock.

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